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Plasma biochemistry and hematology reference values of captive panther chameleons (*Furcifer pardalis*) with special emphasis on seasonality and gender differences

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Meiner Familie

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Abstract

Blood samples of 86 captive panther chameleons (*Furcifer pardalis*) were collected in January and August from the ventral coccygeal vein in order to establish reference intervals of clinical healthy individuals under similar husbandry conditions for plasma biochemistry and hematology for this species. Significant differences were found in phosphorus, glucose, total protein, albumin and white blood cell count between males and females in both seasons. Calcium, alanine aminotransferase, aspartate aminotransferase and lactate dehydrogenase varied in only one season between genders. Significant differences between summer and winter values were present in both genders for uric acid, calcium, phosphorus, glucose, total protein, creatine kinase and albumin. Additionally, females showed seasonal variations for alanine aminotransferase and aspartate aminotransferase, whereas packed cell volume varied in males. Gravid females had significantly higher body-weights and increased values for uric acid, calcium, phosphorus, alanine aminotransferase, total protein and albumin. Cytomorphologic characteristics of blood cells in stained blood films were evaluated to serve as additional parameters for hematology.

Zusammenfassung

Von 86 in Terrarienhaltung befindlichen Pantherchamäleons (*Furcifer pardalis*) wurden im Januar und August Blutproben aus der ventralen Schwanzvene entnommen, um Referenzintervalle von unter ähnlichen Haltungsbedingungen lebenden, klinisch gesunden Individuen für Blutchemie und Hämatologie zu erstellen. Signifikante Unterschiede zwischen männlichen und weiblichen Tieren fanden sich zu beiden Jahreszeiten für Phosphor, Glukose, Gesamteiweiß, Albumin und Zahl der weißen Blutkörperchen. Kalzium, Alanin-Aminotransferase, Aspartat-Aminotransferase und Laktatdehydrogenase unterschieden sich nur in einer Jahreszeit zwischen den Geschlechtern. Bei beiden Geschlechtern konnten signifikante Unterschiede zwischen Sommer- und Winterwerten bei Harnsäure, Kalzium, Phosphor, Glukose, Gesamteiweiß, Kreatinkinase und Albumin festgestellt werden. Des Weiteren zeigten Weibchen saisonale Veränderungen der Alanin-Aminotransferase und Aspartat-Aminotransferase, und Männchen beim Hämatokrit. Trächtige Weibchen hatten signifikant höhere Körpergewichte und erhöhte Harnsäure-, Kalzium-, Phosphor-, Alanin-Aminotransferase-, Gesamteiweiß- und Albumin-Werte. Als zusätzlicher Parameter in der Hämatologie wurden die zytomorphologischen Charakteristika von Blutzellen in gefärbten Blutausstrichen interpretiert.

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PLASMA BIOCHEMISTRY AND HEMATOLOGY REFERENCE VALUES OF CAPTIVE PANTHER CHAMELEONS (*FURCIFER PARDALIS*) WITH SPECIAL EMPHASIS ON SEASONALITY AND GENDER DIFFERENCES

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Abstract: Blood samples of 86 captive panther chameleons (*Furcifer pardalis*) were collected in January and August from the ventral coccygeal vein in order to establish reference intervals of clinical healthy individuals under similar husbandry conditions for plasma biochemistry and hematology for this species. Significant differences were found in phosphorus, glucose, total protein, albumin, and white blood cell count between males and females in both seasons. Calcium, alanine aminotransferase, aspartate aminotransferase, and lactate dehydrogenase varied in only one season between genders. Significant differences between summer and winter values were present in both genders for uric acid, calcium, phosphorus, glucose, total protein, creatine kinase, and albumin. Additionally, females showed seasonal variations for alanine aminotransferase and aspartate aminotransferase whereas packed cell volume varied in males. Gravid females had significantly higher body weights and increased values for uric acid, calcium, phosphorus, alanine aminotransferase, total protein, and albumin. Cytomorphologic characteristics of blood cells in stained blood films were evaluated to serve as additional parameters for hematology.

Key words: Furcifer pardalis, gender differences, hematology, panther chameleon, plasma biochemistry, seasonality.

INTRODUCTION

Panther chameleons (*Furcifer pardalis*) are endemic to Madagascar.[21] Their habitat is characterized by two seasons, with a rainy season during southern summer (November to March) and a dryer season in winter (April to October).[33] Mating takes place mainly during the rainy season.[33] Adult males reach total lengths of 50 cm while females grow up to 35 cm.[21] Because, as compared to other chameleons, panther chameleons are more adaptable, colorful, easy to breed and impose moderate husbandry requirements, they have been one of the most popular chameleon species kept ex situ for years.[32] In captivity, reproduction usually takes place throughout the whole year with a peak in northern summer months.[33] With increasing numbers of captive chameleons, veterinarians face the challenge to diagnose diseases.[27,29] Hematology and blood chemistry reference values exist for several commonly kept reptile species but are almost completely lacking for the panther chameleon.[2,22,42] The only existing panther chameleon reference values have been collected by the International Species Information System (ISIS) from individuals of unknown background.[28] Investigations in other reptiles indicate large differences between blood chemistry and hematology values of different species

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Table 1. Mean ± standard deviation of data from clinically healthy captive panther chameleons (*F. pardalis*) in this study, between the genders within a season.

Gender	*n*[a]	Age		Body weight (g)		SVL (cm)		Gravid	
		Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer
Male	38	2 yr 2 mo ± 1 y 7 mo	2 yr 9 mo ± 1 yr 6 m	117 ± 47	121 ± 40	16.7 ± 2.3	17.0 ± 2.2	-	-
Female	48	1 yr 8 mo ± 1 yr	2 yr 4 mo ± 1 yr	48 ± 13	54 ± 13	12.3 ± 1.4	12.5 ± 1.3	8	12

[a] *n*, number of animals; SVL, snout-vent-length.

as well as intraspecific differences depending on season and gender.[7,14,34,42] The aim of this study was to establish reference values in the panther chameleon with special emphasis on differences between genders and seasons.

MATERIALS AND METHODS

Blood was collected from 86 animals from five private German collections in January and August 2014 during regular health checkups. Additionally gained data included information concerning general housing (terrarium size, type and size of ventilation, planting, ground material), microclimate (quality, duration, and seasonal differences of lighting and temperature) and nutrition (feeders, amounts fed, vitamin-mineral supplementation, water supply). Ultraviolet bulbs were measured via Solarmeter 6.5 (Solartech Inc., Harrison Township, MI 48045, United States) to assure sufficient UV-B radiation according to manufacturer recommendations.[15,16,19] Chameleons kept under suboptimal conditions were excluded from the study. Hatching date, gender, snout-vent-length, body weight, ovipositions, matings and gravidities were recorded (Table 1) and pictures were taken of head and body. Follow-up phone calls to owners were made regarding health status during the research period. Panther chameleons showing clinical signs of disease, such as bite marks or mouth rot, in a time frame of 4 wk before and 4 wk after blood sampling were excluded from the study.

Blood samples were taken from the ventral coccygeal vein using a heparinized 20-ga needle under manual restraint of the head and hip by a second person. Blood was collected into 0.5 ml lithium-heparin tubes.[4,10,23] Plasma samples for biochemistry were prepared by centrifugation in Hettich EBA 20 centrifuge (Hettich AG, 8806 Bäch, Switzerland) at 2,621 *g* for 10 min.

Plasma was frozen at -18°C in Eppendorf vials and transported on dry ice to IDEXX Laboratories (71636 Ludwigsburg, Germany) within 6 days.

Uric acid (UA), calcium (Ca), phosphorus (P), alanine aminotransferase (ALT), aspartate aminotransferase (AST), glucose (Glu), blood urea nitrogen (BUN), total protein (TP), albumin (Alb), lactate dehydrogenase (LDH) and creatine kinase (CK) were analyzed with a Beckman Coulter AU5800 chemistry analzyer (Beckman Coulter GmbH, 47807 Krefeld, Germany). A trial with 19 plasma samples was made to ascertain comparability of biochemistry between samples frozen at -18°C for a minimum of 7 days and fresh plasma samples. Maximum deviations for precision and accuracy of the methodology applied were within acceptable limits.[17]

Two native blood films per chameleon were made directly after sampling and stained with Diff-Quik® (Medion Grifols Diagnostics AG, 3186 Düdingen, Switzerland) for white blood cell differentiation. At least 400 cells per slide were

Table 2. Comparison of biochemistry measurements from 19 duplicate fresh or frozen plasma samples (-18°C for 6 days) in clinically healthy captive panther chameleons (*F. pardalis*). ALT = alanine aminotransferase; AST= aspartate aminotransferase; BUN = blood urea nitrogen; LDH = lactate dehydrogenase.

Analyte	Mean values		Difference	*P*-values
	Fresh samples	Frozen samples		
Uric acid (mg/dl)	10.2	10.5	2.9%	0.003
Calcium (mmol/L)	2.6	2.7	3.8%	0.261
Phosphorus (mmol/L)	1.9	2.0	5.3%	0.002
ALT (IU/L)	6.7	6.9	2.9%	0.465
AST (IU/L)	12.7	17.4	37.0%	0.001
Glucose (mg/dl)	254.5	247.8	2.6%	0.023
BUN (mg/dl)	1.8	2.2	22.2%	0.010
Total protein (g/dl)	4.94	4.75	3.8%	0.003
Albumin (g/dl)	2.23	2.16	3.1%	<0.001
LDH (U/L)	194.7	208.2	6.9%	0.028
Creatine kinase (U/L)	259.5	391.7	50.9%	0.005

differentiated using the x100 oil objective, and the mean value of both slides was calculated. For identification, the general description of reptilian blood cells and terminology was used. [4,18,38-40] Cell sizes were calculated by measuring length and width of 50 cells of 20 animals in a hemocytometer and calculating the arithmetic average for each (except the cell type referred to as an eosinophil, of which only a few cells were found).

Packed cell volume (PCV) was measured by microhematocrit centrifuge (Hettich Hämatokrit 210, Hettich AG) at 9,529 *g* for 5 min. White and red blood cell (WBC, RBC) counts of cooled blood were obtained using Natt and Herrick's solution in a hemocytometer (Neubauer improved chamber, LO Laboroptik, 61381 Friedrichsdorf, Germany) within six hours. Each blood sample was mixed thoroughly immediately before preparing the hemocytometer.

For statistical comparisons between seasons, only individuals with paired blood samples were used. Reference Value Advisor (National Veterinary School, 87614 Toulouse, France) was used to obtain reference values.[20] A Kolmogorov-Smirnov test was used to test Gaussian distribution, appropriate parametric or nonparametric tests were used to compare groups. Results are displayed as means ± standard deviation and reference intervals. All statistical evaluations were performed in SPSS (21.0, SPSS Inc. Chicago, IL). The significance level was set to 0.05.

RESULTS

Thirty-eight male and 48 female panther chameleons were included in this study. They originated from five different private collections. All values regarding housing conditions were statistically evaluated for housing-related variables, but no significant differences between the five collections were found. Two females died from being egg-bound but had been determined as healthy at the first sampling due to the lack of clinical signs both at examination prior to venipuncture and during monitoring for a long period of time after the sampling.

Sampling was uneventful with the exception of one male panther chameleon, which experienced hemipenal prolapse 8 days after sampling and required amputation. Unilateral or complete skin darkening of the tail behind the injection site developed in most animals and disappeared within several weeks. In a few cases the color change was still visible after six months.

Comparison of biochemistry measurements from the 19 duplicate fresh or frozen plasma samples are summarized in Table

Table 3. Mean ± standard deviation (lower and upper limit of reference interval; *n*) for plasma biochemistry in clinically healthy captive panther chameleons (*F. pardakus*). Winter = January; summer = August; ALT = alanine aminotransferase; AST = aspartate aminotransferase; BUN = blood urea nitrogen; LDH = lactate dehydrogenase.[a]

Analyte	Winter		Summer	
	Male	Female	Male	Female
Uric acid (mg/dl)	8.66 ± 1.3^{A} (2.2 – 21.2; 37)	8.43 ± 4.9^{A} (0.3 – 18.5; 44)	5.47 ± 3.8^{B} (0.0 – 11.8; 36)	4.96 ± 3.1^{B} (0.0 – 11.0; 36)
Calcium (mmol/l)	2.8^{Aa} ± 0.3 (2.2 – 3.5; 36)	5.2 ± 4.6^{Ab} (1.3 – 23.9; 45)	2.4 ± 0.3^{B} (1.7 – 3.1; 36)	3.5 ± 0.9^{B} (1.3 – 10.7; 39)
Phosphorus (mmol/l)	2.0^{Aa} ± 0.4 (1.1 – 2.8; 35)	3.0 ± 1.7^{Ab} (0.9 – 7.9; 45)	1.9 ± 0.3^{Ba} (1.2 – 2.5: 37)	2.1^{c} ± 0.5^{Bb} (1.0 – 3.5; 38)
ALT (IU/l)^{aa}	4.1 ± 1.4 (1.1 – 7.0; 33)	5.0 ± 2.2^{A} (2.0 – 10.0; 43)	5.2 ± 2.6^{a} (0.0 – 10.2; 37)	7.1 ± 1.1^{Bb} (1.1 – 19.2; 33)
AST (IU/l)^{bb}	17.7 ± 0.5 (5.0 – 49.3; 33)	22.3 ± 10.6^{A} (0.0 – 43.2; 45)	18.9 ± 3.1^{a} (4.1 – 48.3; 34)	37.3 ± 2.3^{Bb} (7.8 – 104.5; 38)
Glucose (mg/dl)	290.7 ± 47.1^{Aa} (193.8 – 384.9; 37)	212.7 ± 86.1^{Ab} (58.6 – 395.5; 44)	270.3 ± 30.9^{Ba} (206.7 – 333.9; 36)	176.1 ± 61.6^{Bb} (50.7 – 300.2; 39)
BUN (mg/dl)^{cc}	1.31 ± 1.5 (0 – 3.9; 37)	1.12 ± 0.9 (0.0 – 3.0; 42)	1.32 ± 1.3 (0 – 3.9; 37)	0.58 ± 0.7 (0.0 – 3.0; 39)
Total protein (g/dl)	5.66 ± 0.2^{Aa} (4.6 – 7.0; 37)	5.27 ± 0.8^{Ab} (3.7 – 6.9; 45)	4.80 ± 0.7^{Ba} (3.3 – 6.2; 37)	4.12 ± 0.8^{Bb} (2.6 – 5.7; 38)
Albumin (g/dl)	2.72 ± 0.3^{Aa} (2.1 – 3.3; 37)	2.31 ± 0.4^{Ab} (1.3 – 3.1; 45)	1.90 ± 0.4^{Ba} (1.1 – 2.8; 36)	1.31 ± 0.4^{Bb} (0.5 – 2.2; 36)
LDH (U/l)^{dd}	205.6 ± 0.6 (42.3 – 670.7; 34)	229.8 ± 157.4 (54.3 – 626.4; 45)	210.3 ± 146.2^{a} (0.0 – 490.1; 36)	270.0 ± 22.8^{b} (45.1 – 637.3; 39)
Creatine kinase (U/l)	243.2 ± 135.8^{A} (0.0 – 501.8; 30)	261.5 ± 128.7^{A}(95.0 – 835.8; 37)	211.4 ± 131.2^{B} (0.0 – 465.2; 35)	117.8 ± 1.2^{B} (51.7 – 415.0; 36)

[a] Within rows, large superscripts represent significant differences between seasons within a gender, and small superscripts indicate significant differences between the genders within a season.

Table 4. Mean ± standard deviation (lower and upper limit of reference interval; *n*) for hematology in clinically healthy captive panther chameleons (*F. pardalis*). Winter = January; summer = August; PCV = packed cell volume; RBC = red blood cell count; WBC = white blood cell count.[a]

Analyte	Winter		Summer	
	Male	Female	Male	Female
PCV (%)^{aa}	32.9 ± 9.1^{A} (14.1 –51.1; 34)	33.6 ± 11.0 (11.1 – 56.0; 32)	26.3 ± 6.1^{B} (13.8 – 38.7; 36)	30.1 ± 7.9 (13.6 – 45.7; 37)
RBC (10^5/µl)^{bb}	10.9 ± 3.0 (4.5 – 16.7; 34)	11.3 ± 2.0 (7.2 – 15.2; 30)	11.2 ± 1.9 (7.3 – 15.1; 36)	9.6 ± 0.4 (6.4 – 13.7; 29)
WBC (10^3/µl)^{cc}	7.3 ± 3.1^{a} (1.0 – 13.6; 34)	10.8 ± 5.1^{b} (0.3 – 21.3; 30)	7.3 ± 2.4^{a} (2.3 – 12.2; 37)	9.9 ± 5.1^{b} (2.9 – 15.9; 35)
Heterophils (%)	23.9 ± 6.3^{A} (11.4 – 36.9; 37)	26.3 ± 7.9 (10.7 – 46.9; 45)	38.1 ± 11.7^{Aa} (10.3 – 32.4; 37)	24.2 ± 6.3^{b} (11.3 – 38.6; 40)
Azurophils (%)	8.8 ± 4.0^{Aa} (0.3 – 16.7; 37)	10.7 ± 3.9^{b} (3.7 – 19.2; 43)	10.6 ± 3.8^{A} (2.8 – 18.1; 36)	11.3 ± 4.4 (2.5 – 20.8; 40)
Basophils (%)	0.0 ± 0.0 (0.0 – 0.0; 37)	0.2 ± 0.2 (0.0 – 0.8; 45)	0.1 ± 0.1 (0.0 – 0.4; 36)	0.2 ± 0.2 (0.0 – 0.8; 40)
Eosinophils (%)	0.0 ± 0.0 (0.0 – 0.0; 37)	0.0 ± 0.0 (0.0 – 0.0; 45)	0.0 ± 0.0 (0.0 – 0.0; 37)	0.0 ± 0.0 (0.0 – 0.0; 39)
Lymphocytes (%)	67.3 ± 7.8 (51.4 – 83.1; 37)	63.2 ± 9.1 (40.8 – 80.4; 43)	64.1 ± 7.7 (51.9 – 83.1; 37)	64.1 ± 7.8 (50.7 – 79.7; 40)
Blasts (%)	0.0 ± 0.0 (0.0 – 0.0; 37)	0.01 ± 0.0 (0.0 – 0.2; 45)	0.0 ± 0.0 (0.0 – 0.0; 37)	0.01 ± 0.1 (0.0 – 0.2; 39)

[a] Within rows, large superscripts represent significant differences between seasons within a gender, and small superscripts indicate significant differences between the genders within a season.

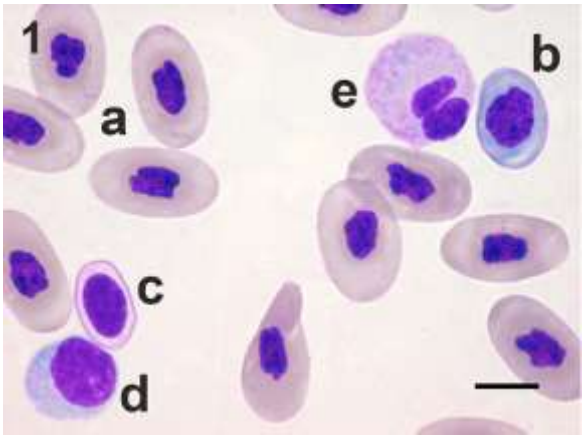

Figure 1. Blood cell differentiation in panther chameleon (*F. pardalis*). Blood smear stained with Diff-Quik (Medion Grifols Diagnostics AG, 3186 Düdingen, Switzerland): (**a**) erythrocytes; (**b**) polychromatic erythrocyte; (**c**) thrombocyte; (**d**) lymphocyte; (**e**) heterophilic granulocyte. Scale bar = 10 µm.

2. Results from hematology and biochemistry values are reported in Tables 3 and 4.

Cytomorphology in stained blood films revealed erythrocytes (Fig. 1), thrombocytes (Fig. 1), mononuclear cells (Figs. 2 and 3), heterophilic granulocytes (Figs. 1, 2 and 4), azurophils (Figs. 5 and 6), basophils (Fig. 7), and a cell type referred to as eosinophil (Fig. 8).

Erythrocytes were characterized by their oval shape of 19.0 ± 0.6 µm in length and 9.0 ± 0.4 µm in width and a centrally positioned, oval, deeply basophilic stained nucleus (Fig. 1a). Few polychromatic or

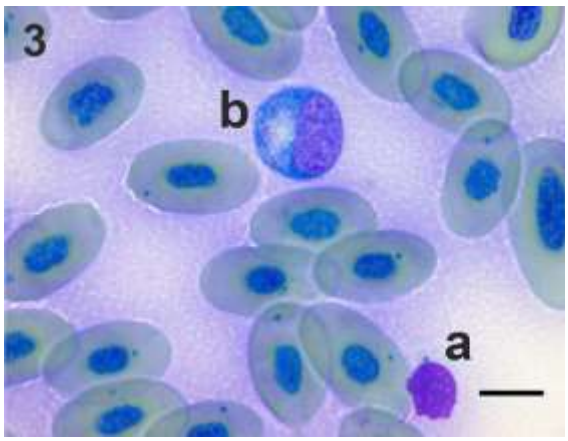

Figure 3. Blood cell differentiation in panther chameleon (*F. pardalis*). Blood smear stained with Diff-Quik (Medion Grifols Diagnostics AG, 3186 Düdingen, Switzerland): (**a**) lysed erythrocyte; (**b**) mono-nuclear cell of plasma cell type with signs of increased cell metabolism (cytoplasmic basophilia, Golgi apparatus well visible as light blue area close to the nucleus). Scale bar = 10 µm.

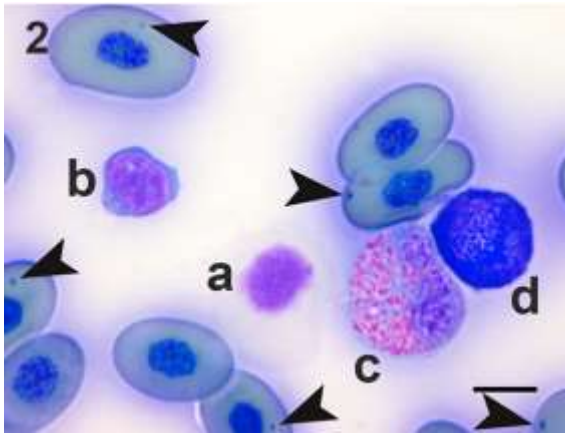

Figure 3. Blood cell differentiation in panther chameleon (*F. pardalis*). Blood smear stained with Diff-Quik (Medion Grifols Diagnostics AG, 3186 Düdingen, Switzerland): (**a**) hemolyzed erythrocyte; (**b**) lymphocyte; (**c**) heterophilic granulocyte; (**d**) azurophil; arrows = punctate intracytoplasmatic structures of unknown significance. Scale bar = 10 µm.

hemolyzed erythrocytes were found among all chameleons (Figs. 1b, 2a, 3a). Mitotic figures in erythrocytes were rarely observed. Small round, deeply basophilic staining structures could be found occasionally in erythrocytes without concurrent signs of disease (Fig. 2 arrows).

Thrombocytes were round to oval-shaped with a diameter of 7.1 ± 1.5 µm and mostly a centrally located nucleus consisting of dense and finely granulated

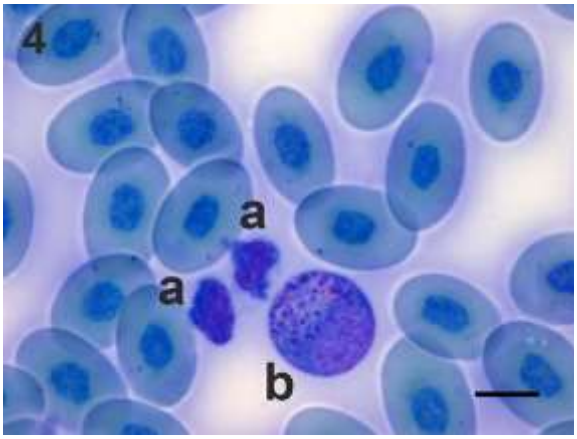

Figure 4. Blood cell differentiation in panther chameleon (*F. pardalis*). Blood smear stained with Diff-Quik (Medion Grifols Diagnostics AG, 3186 Düdingen, Switzerland): (**a**) two small lymphocytes; (**b**) granulocyte resembling a heterophilic metamyelocyte characterized by cytoplasmic basophilia, a nonlobulated nucleus, and the concurrent presence of immature, round, basophilic primary granules and mature, ellipsoid, eosinophilic secondary granules. Scale bar = 10 µm.

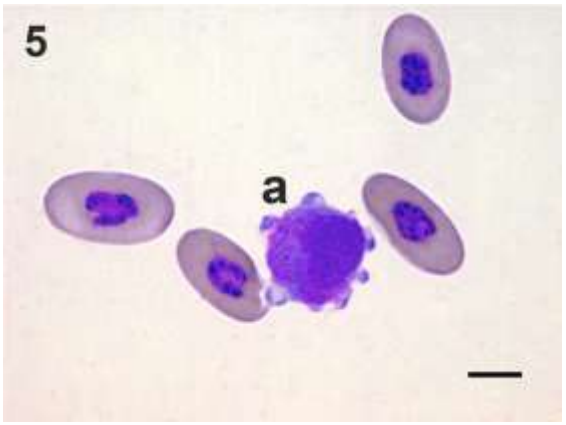

Figure 5. Blood cell differentiation in panther chameleon (*F. pardalis*). Blood smear stained with Diff-Quik (Medion Grifols Diagnostics AG, 3186 Düdingen, Switzerland): (**a**) azurophil with cytoplasmatic bleb formaton. Scale bar = 10 µm.

chromatin (Figs. 1c, 7a). The cytoplasm was colorless to greyish-basophilic in contrast to the clearly basophilic cytoplasm of small lymphocytes and often contained one or more small punctate pole-bodies closely positioned to the nucleus. Thrombocytes tended to aggregate and often formed cell clusters.

Concerning the mononuclear cells with a clear, non-granulated cytoplasm, both lympho- and monocytoid types could be found. Lymphocytes, the predominant leukocytes in panther chameleons, were spherically shaped with a diameter of 9.4 ± 4.1 µm, with the maximum size of these cells being 25.0 µm (Figs. 1d, 2b, 4a, 6a,

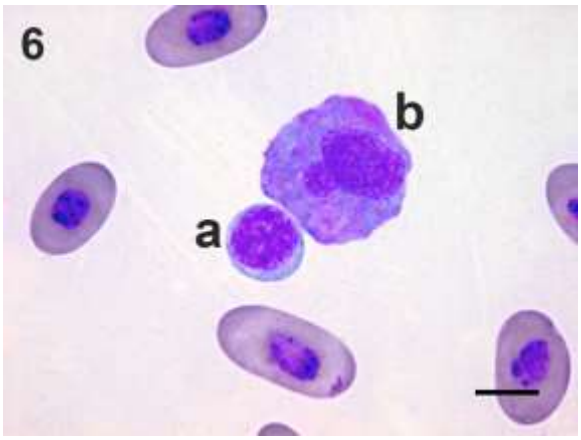

Figure 6. Blood cell differentiation in panther chameleon (*F. pardalis*). Blood smear stained with Diff-Quik (Medion Grifols Diagnostics AG, 3186 Düdingen, Switzerland): (**a**) lymphocyte; (**b**) enlarged azurophil of the sample in Figure 5. Scale bar = 10 µm.

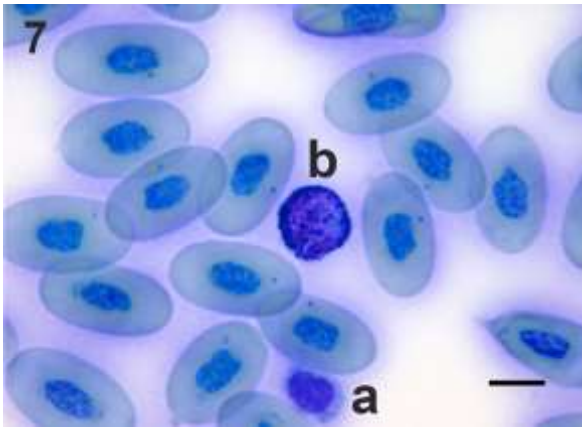

Figure 7. Blood cell differentiation in panther chameleon (*F. pardalis*). Blood smear stained with Diff-Quik (Medion Grifols Diagnostics AG, 3186 Düdingen, Switzerland): (**a**) thrombocyte; (**b**) basophilic granulocyte with prominent basophilic granulation (blackberry type). Scale bar = 10 µm.

8a). Most of the lymphocytes contained a large nucleus with coarse chromatin, leaving only a small band of cytoplasm visible around it. Small lymphocytes were sometimes hard to differentiate from thrombocytes, especially within the hemocytometer. Large mononuclear cells occurred in almost every blood film and were classified as either large lymphocytes or monocytes following a cytomorphologic differentiation scheme to evaluate cell size, nucleo-cytoplasmic ratio, color and structure of cytoplasm as well as chromatin density, shape, and position of the nucleus. Some of the large mononuclear cells presented with signs of reactivity such as a

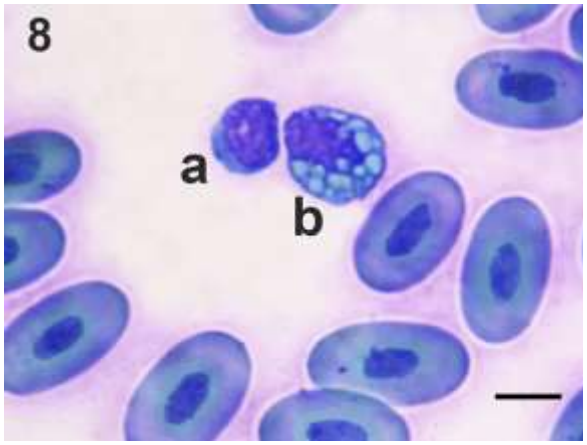

Figure 8. Blood cell differentiation in panther chameleon (*F. pardalis*). Blood smear stained with Diff-Quik (Medion Grifols Diagnostics AG, 3186 Düdingen, Switzerland): (**a**) lymphocyte; (**b**) cell type referred to as eosinophilic granulocyte. Scale bar = 10 µm.

deeply basophilic cytoplasm with a well visible Golgi apparatus close to the nucleus. Occasionally, these cells resembled mammalian plasma cells with an ellipsoid shape and an eccentrically located nucleus (Fig. 3b).

Heterophilic granulocytes had a spherical shape with an eccentric, mostly lobed nucleus containing clumpy, basophilic-purplish chromatin (Figs. 1e, 2c). Cytoplasm was pale blue to colorless and filled with fine, rod-shaped eosinophilic granules, which were more or less visible depending on the individual stain. Cell size averaged 17.5 ± 1.5 µm in diameter, but could increase to 35 µm in diameter, the latter of which was observed in females after egg laying. Occasionally, cells with characteristics of metamyelocytes were present (Fig. 4b).

Azurophils in general appeared as deeply basophilic cells averaging 16.5 ± 0.8 µm in diameter with a high nucleocytosomal ratio (Fig. 2d). The dark purple nucleus was round or kidney shaped with a dense, fine chromatin pattern. Occasionally, signs of activation such as cytoplasmatic bleb formations were present (Fig. 5a). Similar to the heterophils, the size of azurophils could increase to almost double size in females after egg laying (Fig. 6b).

Basophils were spherically shaped with an average diameter of 16.0 ± 0.9 µm. In comparison to azurophils, they were usually smaller. The nucleus was often hard to determine and of variable shape. Cytoplasm stained deeply basophilic with spherical, large, blackberry-like granules (Fig. 7b). A typical staining artefact in most basophils was alcohol dependent lysis of the granules, which resulted in a pale stained cytoplasm, occasionally with a basophilic stained circle around the cell.

An unusual cell type found extremely rarely was referred to as eosinophil (Fig. 8b). This blood cell had a diameter of 15.0 ± 0 µm with a variably positioned nucleus containing dense chromatin. The cytoplasm was bright basophilic and contained large, pale-blue, more or less spherical granules.

The analytes of Glu, TP and Alb showed significantly higher values in males than in females (Table 3). The analytes of Ca, P, ALT, AST, LDH and WBC were significantly higher in females (Tables 3 and 4). Gravid females were included in these evaluations.

In both genders, UA, Ca, P, TP and Alb values were significantly higher in winter than in summer (Table 3). In females, Glu was significantly higher in winter than in summer, and ALT, AST, CK and RBC had higher values in summer (Tables 3, 4). Males had significantly higher CK, heterophilic granulocytes counts and PCV in winter but lower Glu and azurophils (Tables 3, 4).

In summer, gravid females (n = 12) had significantly higher body weights than did non-gravid females (57.6 ± 7.8 vs. 48.2 ± 13.1 g, $P = 0.026$), UA (10,163.6 ± 6,802.6 vs. 5,040.2 ± 2,917.2 mg/dl, $P = 0.028$), Ca (5.3 ± 3.5 vs. 2.9 ± 2.9 mmol/l, $P = 0.036$), P (2.6 ± 1.1 vs. 1.9 ± 0.6 µmol/l, $P = 0.062$), ALT (30.5 ± 28.1 vs. 9.5 ± 10.6 IU/l, $P < 0.001$), TP (4.7 ± 1.1 vs. 3.9 ± 0.9 g/dl, $P = 0.019$) and Alb (1.8 ± 0.6 vs. 1.2 ± 0.4 g/dl, $P = 0.001$). In winter, there was no significant difference between gravid and nongravid females.

DISCUSSION

Limitations of this study were number of animals and the size of some smaller animals, which restricted blood volume and possible investigations.[8,17,35] Physiological reference ranges are defined as the 95% confidence interval of all measurements, and recommended representative ‘n’ includes a minimum of 40 individuals of the same species.[17,35] All reference values of females in winter in the present study reach this limit, but the other reference values range between 30 and 40 individuals. For these cases, reference interval is considered to be the observed range of values that remains after

elimination of outliers, which makes reference intervals less reliable.[17] Comparability and interpretation of the obtained hematology and plasma biochemistry values to previously published data has to be treated with caution as well, as laboratory findings in ectothermic animals are strongly influenced by extrinsic and intrinsic factors, such as husbandry, gender, age, nutritional status, reproductive status, temperature, season and analytical procedure.[34-36,38,41] Inclusion of several facilities was necessary because availability of the species, maintenance costs, legal requirements and the need of one cage per individual restricts the opportunities in Germany to keep a sufficient number of chameleons under identical experimental conditions.

One male panther chameleon experienced hemipenal prolapse 8 days after sampling. A possible cause could have been that the needle was inserted too close to the hemipenal muscles in this case, although the point of insertion was behind the hemipenal pockets at ¼ to ½ of tail length as suggested in the literature.[39] Other males with similar sampling location showed no reaction. To safely avoid the hemipenal pockets and corresponding muscles in panther chameleons, the authors recommend inserting the needle at least 6 cm behind the hemipenal pockets in adult panther chameleons.

The effect of freezing for 6 days at -18°C was evaluated on 19 samples (Table 2). The highest differences were measured for AST and CK (12.7 ± 6.3 vs. 17.4 ± 8.5 IU/l, p P = 0.001 for AST, 259.5 ± 211.6 vs. 391.7 ± 371.7 U/l, P = 0.010 for CK). Although statistically significant in some cases, the other analytes differences were considered not to be clinically relevant. Using frozen plasma samples is a standard procedure in reptiles.[24,37] Nevertheless, it is recommendable to compare the observed reference values only to samples that underwent similar conditions.

Morphology of blood cells in this study mostly resembled previous descriptions in reptiles, specifically those for panther chameleons, flap-necked chameleons (*Chamaeleo dilepis*) and non-chameleon species such as green iguanas (*Iguana iguana*) or bearded dragons (*Pogona vitticeps*).[22,25,26,40,42] As reported for other lizards, lymphocytes were the predominant leukocyte found in the blood films.[22,40]

Similar structures to the small, deeply basophilic staining structures that were found occasionally in erythrocytes (Fig. 2 arrows) are known to occur regularly in other reptile erythrocytes.[40] Due to the fact that they are usually seen in healthy reptiles, these are considered non-pathologic or staining artefacts.[40]

Heterophils and azurophils with increased cell size were found in many blood smears. An increase of cell size has been reported in thrombocytes of Houbara bustards (*Chlamydotis undulata macqueeni*) for certain pathologic conditions, such as chronic inflammations, thus supporting the thesis that different cell sizes of leukocytes may be of diagnostic value for interpreting hemograms in panther chameleons.[11] A link to gravidity-related cell size increase has not been shown in any other study yet. Because all slides of the affected chameleons revealed the same phenomenom - and slides of nongravid individuals processed in the same batch were free of them - staining artefacts are improbable. Further research is needed to reveal exact function and origin of the increased cell sizes in panther chameleons.

The cell type referred to as eosinophil granulocyte resembles eosinophils descrybed for *I. iguana* and yellow-headed temple turtles (*Hieremys annandalii*) but differs strongly from those described for other reptiles.[5,22] In general, the existence and function of eosinophils in reptiles is poorly investigated.[38,40] Occurrence is variable in squamates, and identification mostly relies on morphologic features.[40] While most eosinophils in reptiles stain positive with benzidine peroxidase, this is not the case in the green iguana[40], raising questions about function and identity of

this cell type. It has to be emphasized that despite identical nomenclature, which has led to extrapolation of functionality of mammalian eosinophils to their reptile counterparts[30], there is no proof that the cell types described in lizards have the same immunologic function. Further studies are needed to function and identity of this cell type.

As in other reptiles, significant differences between male and female panther chameleon blood values were found.[7,37,41] Glucose, TP and Alb levels were higher in males compared to females. A similar elevation of Glu in males was found in pancake tortoises (*Malacochersus tornieri*), but no reason has been found yet.[37] Other differences between genders might be primarily related to folliculogenesis, during which vitellin is released into blood and causes elevated Ca, P, TP and Alb levels for building egg yolk and shells.[43] Higher ALT, AST and LDH enzyme levels in females are known from Asian yellow pond turtles (*Ocadia sinensis*) and *P. vitticeps* as well, and are suggested to be caused by either muscle injuries during matings, higher activity in general or physiologic hepatic lipidosis before oviposition.[7,42] Other possible causes include more severe tissue trauma occurring during venipuncture in females due to their smaller body size.[13]

Finding of follicuogenesis-related values in females corresponds to the differences found between gravid and non-gravid panther chameleons in this study. In addition to Ca, P, TP and Alb, all gravid females in our study showed higher values in UA and ALT. Significant differences between gravid and nongravid females are restricted to summer season, probably because there were fewer gravid females in winter. Additionally, the gravid females in winter were likely in earlier stages of gravidity.

Significant differences were found between seasons. This has been reported mainly for species holding torpor during winter.[2,6,14] It is remarkable that such differences can also be found in panther chameleons, which only show lower activity levels during colder season but no torpor. A similar finding has been stated in other lizards with less activity in winter such as *P. vitticeps*.[42]

Uric acid is known to increase during winter in tortoises and lizards.[6,14,42] Analytes of TP and Alb increased in tortoises during winter, which might be related to mild dehydration.[1] Calcium and P might increase in winter due to a lower UV-B supply as compared with summer, when most animals of this study were kept outside. Lack of UV-B causes a lack of vitamin D3 production in the skin, which stimulates hormones to mobilize Ca from bone in order to maintain normal blood levels.[30] Additionally, owners tended to supplement more Ca in winter. Folliculogenesis seems to play no role in this case because more females were gravid in summer and values of Ca were increased in males during winter as well.

Glucose decreases in winter in males may result from temperature and lower food intake, as suggested by different authors in studies of tortoises, turtles and lizards.[1,6,7,14,43] In contrast, it is difficult to explain why Glu is higher in females in winter than in summer. Relationship to folliculogenesis seems unlikely because fewer females were gravid in winter. In summer, ALT and AST, both enzymes found in liver and muscle cells, may increase due to warmer temperatures and thereby higher metabolic activity, as also stated in other reptiles.[1,6,7]

Reasons for the increased values of heterophilic granulocytes and azurophils during winter refer to the relative counts. Due to lowered metabolism activity of the ectothermic chameleons in winter, one can expect decreased blood plasma volume or a physiologic lymphopenia and therefore relatively elevated heterophilic granulocytes and azurophils.[40]

Uric acid in panther chameleons was higher than in most other reptile species during winter, which might have been

caused by storage of nitrogenous waste during slower metabolism period, as suggested before.[6] Varying feeding time of each individual panther chameleon could play another role.[43] No fasting period prior to sample collection was determined, resulting in different stages of purine and pyrimidine degradation in the studied panther chameleons and, therefore, varying release of uric acid from liver into blood. Wild, healthy common chameleons (*Chamaeleo chamaeleon*) also showed lower UA values in one single study, and ISIS values of panther chameleons showed more than two-times lower UA values than the results of this study.[10,28]

Calcium values in panther chameleons were lower than reported for other reptiles, but comparable to values in three wild caught *Gallotia* species lizards as the only reptile (except other chameleon species) known to show such low Ca levels.[3,31] Only some female panther chameleons in this study showed higher Ca values, probably due to folliculogenesis and mobilization of bone Ca.[43] The finding of lower general Ca levels is in concordance with data collected from 35 *F. pardalis* of eight zoological institutions in ISIS.[28] Another study about Ca levels in captive veiled chameleons (*Chamaeleo calyptratus*) revealed similar results: Young veiled chameleons with histologically proven healthy bones showed low Ca values similar to the adult healthy panther chameleons of this study.[24] Hence, it may be assumed that Ca values in chameleons are generally lower than in other lizard species, at least in captivity.[24] According to postmortem findings by the first author (AL), subclinical metabolic bone disease linked with developing nonphysiological bone structure at adult age has to be included in the list of differentials. In contrast to wild-caught chameleons, many captive-bred chameleons typically reveal signs of mild metabolic bone diseases (Laube, pers. obs.). No statistically significant differences in any values between wild caught and captive bred panther chameleons could be found in the present study, but the examined population contained only eight wild-caught individuals in total, and all had been kept by their present owners for at least 9 mo before first sampling. Further studies should be done to assess whether low Ca levels are a finding of captive-kept chameleons due to suboptimal husbandry or whether this also occurs in wild chameleons.

The AST in these panther chameleons reference values was lower than for reptiles in general, wild *C. chamaeleon* and *F. pardalis* from zoos.[3,10,28] Elevated AST levels in female panther chameleons with hepatic lipidosis, compared with lower levels of the healthy panther chameleons in this study, recommend this parameter as a diagnostic tools to help in the diagnosis of hepatic diseases.[27]

Glucose reference values in panther chameleons were elevated relative to those known from other reptiles.[3] Several authors reported high Glu in context of diseases as suggested in a case report with a warty chameleon (*Furcifer verrucosus*) suffering from nephritis and in a female *F. pardalis* suffering from hepatic lipidosis, although the authors of the latter study do not exclude physiologic lipidosis.[10,27,29] Hyperglycemia has also been reported in reptiles with pancreatitis.[29] Because Glu was comparably high in studies with clinically healthy wild *C. chamaeleon*, disease as the only reason for elevated Glu appears doubtful. In the present study, chameleons showing signs of disease before, during and weeks after venipuncture were excluded from reference values. Therefore it is unlikely that subclinically that subclinical disease caused high Glu. In other reptiles, high Glu levels have also been associated with stress or trauma.[3,31] However, stress induced by handling the animals appears to be an unlikely reason, as wild animals which were not accustomed to handling showed lower Glu values in other reptiles.[6,37] Furthermore, even some apparently

exceptionally tame panther chameleons of this study had high Glu levels.

Packed cell volume in this study showed lower and higher limits of reference intervals than are seen in reptiles in general and earlier in *F. pardalis*, but correspond to a study with panther chameleons with hepatocellular lipidosis.[3,27,28] Common reference values for reptiles suggest anemia in PCV values lower than 20%.[3] Several panther chameleons of our data set had lower PCV but were free from any signs of anemia, e.g. weakness, pale mucous membranes, or dehydration. The vast majority of samples only showed a relative low or high PCV. Absolute low PCVs were restricted to few specimens free of any clinical signs of anemia and diseases. From this it can be concluded that hemodilution by lymph contamination or measuring errors due to very small hematocrit tubes are the most probable cause for low PCV, at least in most cases.[38,41] Overheparinization seems not to play a role in this case, all blood samples were handled the exact same way and too low or high PCVs were restricted to certain samples.

The WBC count in panther chameleons differed from values for reptile in general as well as from wild *C. chamaeleon* but were comparable to *F. pardalis* values from several zoos.[3,10,28] White blood cell counts have wide ranges in many reptiles and strongly depend on counting method, user skills and intraspecific differences.[12,38] Natt and Herrick's method, especially, is known to result in a high variability in white blood cell count compared to other systems, with coefficients of variation up to 17.2% in one study with birds, but has the advantage that it allows WBC and RBC to be counted in the same hemocytometer.[12,41]

Differential counts in panther chameleons of zoos and another study show much higher values for basophils.[27,28] Possible causes for this difference other than true elevations include divergent staining properties, uneven distribution of basophils within the counted area of the blood film, and insufficient numbers of differentiated cells.[41]

In conclusion, differences between various species in hematology and blood chemistry emphasize the importance of species- and gender-specific reference values in reptiles.[38,39,41] The present study offers guidelines for hematology and blood chemistry of healthy captive panther chameleons. This should encourage and help veterinarians in the field and in practice who are confronted with diagnostic work-up in an individual of this commonly kept reptile species.

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LITERATURE CITED

1. Anderson NL, Wack RF, Hatcher R. Hematology and clinical chemistry reference ranges for clinically normal, captive New Guinea snapping turtle (*Elseya novaeguineae*) and the effects of temperature, sex and sample type. J Zoo Wildl Med. 1997;28:394-403.

2. Andreani G, Carpenè E, Cannavacciuolo A, Di Girolamo N, Ferlizza E, Isani G. Reference values for hematology and plasma biochemistry variables, and protein electrophoresis of healthy Hermann's tortoises (*Testudo hermanni ssp.*). Vet Clin Path. 2014;43:573-583.

3. Campbell T. Interpretation of the reptilian blood profile. Exot Pet Pract. 1998;3:33-36:

4. Campbell TW, Ellis CK. Avian and exotic animal hematology and cytology. 3rd ed. Iowa: Blackwell Publishing; 2007. Pp. 287.

5. Chansue N, Sailasuta A, Tangtrongpiros J, Wangnaitham S, Assawawongkasem N. Hematology and clinical chemistry of adult yellow-headed temple turtles (*Hieremys annandalii*) in Thailand. Vet Clin Path. 2011;40:174-184.

6. Christopher MM, Berry KH, Wallis IR, Nagy KA, Henen BT, Peterson CC. Reference intervals and physiologic alterations in hematologic and biochemical values of free-ranging desert tortoises in the Mojave desert. J Wildl Dis. 1999;35:212-238.

7. Chung CS, Cheng CH, Chin SC, Lee AH, Chi CH. Morphologic and cytochemical characteristics of Asian yellow pond turtle (*Ocadia sinensis*) blood

cells and their hematologic and plasma biochemical reference values. J Zoo Wildl Med. 2009;40:76-85.

8. Cray C. Reference intervals in avian and exotic hematology. Vet Clin Exot Anim. 2014;18:105-116.

9. Cuadrado M, Molina-Prescott I, Flores L. Comparison between tail and jugular venipuncture techniques for blood sample collection in common chameleons (*Chamaelo chamaeleon*). Vet J. 2003;166:93-97.

10. Cuadrado M, Díaz-Paniagua C, Quevedo MA, Aguilar JM, Molina Prescott I. Hematology and clinical chemistry in dystocic and healthy post-reproductive female chameleons. J Wildl Dis. 2002;38:395-401.

11. D'Aloia MAE, Samour JH, Howlett JC. Haemopathological responses to chronic inflammation in the Houbara bustard (*Chlamydotis undulata macqueenii*). Comp Haematol Int. 1994;4:203-206.

12. Dein FJ, Wilson A, Fischer D, Langenberg P. Avian leucocyte counting using the hemocytometer. J Zoo Wildl Med. 1994;25:432-437.

13. Divers SJ, Redmayne G, Aves EK. Haematological and biochemical values of 10 green iguanas (*Iguana iguana*). Vet Rec. 1996;138:203-205.

14. Erler M. Saisonale Veränderungen hämatologischer und blutbiochemischer Werte bei europäischen Landschildkröten (*Testudo graeca*, *Testudo hermanni*, *Testudo marginata*). [Seasonal changes of hematological and blood biochemistry values in European tortoises (*Testudo graeca*, *Testudo hermanni*, *Testudo marginata*)]. Doctoral thesis, Institute of Zoology, Fishery Biology and Fish Diseases of the Veterinary Faculty, Ludwigs-Maximillians-University. Munich: 2003.

15. Ferguson GW, Gehrmann WH, Chen TC, Dierenfeld ES, Holick MF. Effects of artificial ultraviolet light exposure on reproductive success of the female panther chameleon (*Furcifer pardalis*) in captivity. Zoo Biol. 2002;21:525-537.

16. Ferguson GW, Jones JR, Gehrmann WH, Hammack SH, Talent LG, Hudson RD, Dierenfeld ES, Fitzpatrick MP, Frye FL, Holick MF, Chen TC, Lu Z, Gross TS, Vogel JJ. Indoor husbandry of the panther chameleon *Furcifer pardalis*: Effects of dietary vitamins A and D and ultraviolet irradiation on pathology and life-history traits. Zoo Biol. 1996;15:279-299.

17. Friedrichs KR, Harr KE, Freeman KP, Szladovits B, Walton RM, Barnhart KF, Blanco-Chavez J. ASVCP reference interval guidelines: determination of de novo reference intervals in veterinary species and other related topics. Vet Clin Path. 2012;41:441–453.

18. Fudge, AM (ed.): Laboratory medicine: Avian and exotic pets. 1st ed. Philadelphia: Saunders; 2000. Pp. 486.

19. Gallenstein, G. Veterinary care. Cham Info Netw. 1998;30:3-13.

20. Geffré A, Concordet D, Braun JP, Trumel C. Reference Value Advisor: a new freeware set of macroinstructions to calculate reference intervals with Microsoft Excel. Vet Clin Path. 2011;40:107-112.

21. Glaw F, Vences M. A field guide to the amphibians and reptiles of Madagascar. 3rd ed. Cologne: Vences & Glaw Verlag; 2007. P. 496.

22. Harr KE, Alleman R, Dennis PM, Maxwell LK, Lock BA, Bennett RA, Jacobson ER. Morphologic and cytochemical characteristics of blood cells and hematologic and plasma biochemical reference ranges in green iguanas. J Am Vet Med Assoc. 2001;218:915-921.

23. Harr KE, Raskin RE, Heard DJ. Temporal effects of 3 commonly used anticoagulants on hematologic and biochemical variables in blood samples from macaws and Burmese pythons. Vet Clin Path. 2005;34:383-388.

24. Hoby S, Wenker C, Robert N, Jermann T, Hartnack S, Segner H, Aebischer CP, Liesegang A. Nutritional metabolic bone disease in juvenile veiled chameleons (*Chamaeleo calyptratus*) and its prevention. J Nutr. 2010;140:1923-1931.

25. Irizarry-Rovira AR, Wolf A, Bolek M, Christian JA, DeNicola DB. Blood smear from a wild-caught panther chameleon (*Furcifer pardalis*). Vet Clin Path. 2002;31:129-132.

26. Jacobson ER, Telford SR. Chlamydial and poxvirus infections of circulating monocytes of a flap-necked chameleon (*Chamaeleo dilepis*). J Wildl Dis. 1990;26:572-577.

27. Jones JR, Ferguson GW, Gehrmann WH, Frye FL. Hematology and serum chemistries of captive-raised female panther chameleons (*Chamaeleo pardalis*), with hepatocellular lipidosis. J Herp Med Surg. 1996;6:10-13.

28. ISIS (International Species Information System). ISIS Species Holdings, 12101 Johnny Cake Ridge Road, Apple Valley; c2008.

29. Knotek Z, Knotková Z, Trnková S, Dorrestein GM, Lewis W. Chronic liver disease and subchronic nephritis in a male warty chameleon (*Furcifer verrucosus*) with transient hyperglycaemia – case report. Acta Vet. 2011;80:397-400.

30. Mader, DR. Reptile medicine and surgery. 1st ed. St. Louis: Saunders; 1996. P. 1242.

31. Martínez Silvestre A, Rodríguez Domínguez MA, Mateo JA, Pastor J, Marco I, Lavín S, Cuenca R. Comparative haematology and blood chemistry of endangered lizards (*Gallotia* species) in the Canary islands. Vet Rec. 2004;155:266-269.

32. Mattioli F, Gili C, Andreone F. Economics of captive breeding applied to the conservation of selected amphibian and reptile species from Madagascar. Natura Soc it sci nat Museo civ Stor nat Milano. 2006;95:67-80.

33. Nečas P. Chameleons: Nature's hidden jewels. 1st ed. Francfort: Edition Chimaira; 1999. P. 343.

34. Origgi FC. Reptile immunology. *In*: Jacobson E. (ed.). Infectious diseases and pathology of reptiles, color atlas and text. Boca Raton: CRC Press; 2007. Pp. 131-166.

35. Pendl H, Kreyenbuehl K. Clinical pathology. *In*: Poland G, Raftery A (eds.) BSAVA Manual of Backyard Poultry. Gloucester: BSAVA Publications; (In press).

36. Pendl H, Tizard I. Immunology. *In*: Speer B (ed). Current veterinary therapy in avian medicine. Atlanta: Elsevier; (In press).

37. Raphael BL, Klemens MW, Moehlman P, Dierenfeld E, Karesh WB. Blood values in free-ranging pancake tortoises (*Malacochersus tornieri*). J Zoo Wildl Med. 1994;25:63-67.

38. Stacy NI, Alleman AR, Sayler KA. Diagnostic hematology of reptiles. Clin Lab Med. 2011;31:87-108.

39. Stahl SJ. Reptile hematology and serum chemistry. *In*: Proceedings of the North American Veterinary Conference; 2006. Pp. 1673-1676.

40. Strik NI, Alleman R, Harr KE. Circulating inflammatory cells. *In*: Jacoboson E. (ed.) Infectious diseases and pathology of reptiles, color atlas and text. Boca Raton: CRC Press; 2007. Pp. 167-218.

41. Sykes JM, Klaphake E. Reptile hematology. Vet Clin Exot Anim. 2015;18:63-82.

42. Tamukai K, Takami Y, Akabane Y, Kanazawa Y, Une Y. Plasma biochemical reference values in clinically healthy captive bearded dragons (*Pogona vitticeps*) and the effects of sex and season. Vet Clin Path. 2011;40:368-373.

43. Wright KM, Skeba S. Hematology and plasma chemistries of captive prehensile-tailed skinks (*Corucia zebrata*). J Zoo Wildl Med. 1992;23:429-432.

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